9-2015

# Comprehensive phenotypic analysis and quantitative trait locus identification for grain mineral concentration, content, and yield in maize (Zea mays L.)

Riliang Gu
*China Agricultural University*

Fanjun Chen
*China Agricultural University*

Bingran Liu
*China Agricultural University*

Xin Wang
*China Agricultural University*

Jinghao Liu
*China Agricultural University*

*See next page for additional authors*

# Comprehensive phenotypic analysis and quantitative trait locus identification for grain mineral concentration, content, and yield in maize (Zea mays L.)

**Abstract**
Biofortification by enhanced mineral density in maize grain through genetic improvement is one of the efficient ways to solve global mineral malnutrition, in which one key step is to detect the corresponding Quantitative Trait Loci (QTL). In this work, a maize recombinant inbred population (RIL) was grown to maturity in four field environments with two locations × two years. Phenotypic data of mineral nutrition concentration, content and yield were determined for grain copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), magnesium (Mg), potassium (K) and phosphorus (P). Analysis of variance (ANOVA) showed significant effects of genotype, location and year for all investigated traits. Location showed the highest effect for all mineral yields, and Zn and Cu content and concentration, while year had the strongest impact for Mn, K, and P content and concentration. Heritabilities (h2) of different traits varied with higher h2 (72-85%) for mineral concentration and content and lower (48-63%) for nutrient yields. Correlation coefficient analysis revealed significant positive correlations for grain concentration between several minerals. P had the closest correlations to other elements, while Cu had the lowest. When environments were analyzed individually, a total of 28, 25, and 12 QTL were identified for nutrient concentration, content and yield, respectively. Among these QTL, 8 QTL were consistent within traits across different environments. These stable QTL may be most promising for controlling mineral accumulation in maize grain. Co-localization of QTL for different traits was found for 12 chromosome regions, suggesting that common processes might contribute seed nutrient accumulation

**Keywords**
maize, nutrient elements, grain quality, quantitative trait locus

**Disciplines**
Agricultural Science | Agronomy and Crop Sciences | Genetics and Genomics | Plant Breeding and Genetics

**Comments**
This is a post-peer-review, pre-copyedit version of an article published in Theoretical and Applied Genetics. The final authenticated version is available online at: https://doi.org/10.1007/s00122-015-2546-5.

**Authors**
Riliang Gu, Fanjun Chen, Bingran Liu, Xin Wang, Jianchao Liu, Pengcheng Li, Qingchun Pang, Jordon Pace, Thomas Lubberstedt, Guohua Mi, and Lixing Yuan

Theoretical and Applied Genetics

# Comprehensive phenotypic analysis and quantitative trait locus identification for seed mineral concentration, content, and yield in maize grains

--Manuscript Draft--

| | |
|---|---|
| **Manuscript Number:** | |
| **Full Title:** | Comprehensive phenotypic analysis and quantitative trait locus identification for seed mineral concentration, content, and yield in maize grains |
| **Article Type:** | Original Paper |
| **Abstract:** | Biofortification by enhanced mineral density in maize grain through genetic improvement is one of the efficient ways to solve global mineral malnutrition, in which one key step is to detect the corresponding Quantitative Trait Loci (QTL). In this work, a maize recombinant inbred population (RIL) was grown to maturity in four field environments with two locations × two years. Phenotypic data of mineral nutrition concentration, content and yield were determined for grain copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), magnesium (Mg), potassium (K) and phosphorus (P). Analysis of variance (ANOVA) showed significant effects of genotype, location and year for all investigated traits. Location showed the highest effect for all mineral yields, and Zn and Cu content and concentration, while year had the strongest impact for Mn, K, and P content and concentration. Heritabilities (h2) of different traits varied with higher h2 (72-85%) for mineral concentration and content and lower (48-63%) for nutrient yields. Correlation coefficient analysis revealed significant positive correlations for grain concentration between several minerals. P had the closest correlations to other elements, while Cu had the lowest. When environments were analyzed individually, a total of 28, 25, and 12 QTL were identified for nutrient concentration, content and yield, respectively. Among these QTL, 8 QTL were consistent within traits across different environments. These stable QTL may be most promising for controlling mineral accumulation in maize grain. Co-localization of QTL for different traits was found for 12 chromosome regions, suggesting that common processes might contribute seed nutrient accumulation. |
| **Corresponding Author:** | Lixing Yuan<br>China Agricultural University<br>Beijing, CHINA |
| **Corresponding Author Secondary Information:** | |
| **Corresponding Author's Institution:** | China Agricultural University |
| **Corresponding Author's Secondary Institution:** | |
| **First Author:** | Riliang Gu |
| **First Author Secondary Information:** | |
| **Order of Authors:** | Riliang Gu |
| | Fanjun Chen |
| | Bingran Liu |
| | Xin Wang |
| | Jianchao Liu |
| | Pengcheng Li |
| | Qingchun Pang |
| | Jordon Pace |
| | Thomas Lübberstedt |
| | Guohua Mi |
| | |

| | |
|---|---|
| | Lixing Yuan |
| **Order of Authors Secondary Information:** | |
| **Author Comments:** | Dear Editor,<br><br>Please find enclosed a manuscript that we would like to submit for consideration of publication in Theoretical and Applied Genetics (TAG) entitled "Comprehensive phenotypic analysis and quantitative trait locus identification for seed mineral concentration, content, and yield in maize grains".<br><br>Enhancement of mineral nutrient density in crop grain, so called as biofortification, is one of the efficient ways to solve global nutrient malnutrition, in which one key step is to detect favorable QTL/genes/alleles. Although several QTL analysis have been conducted to analyze mineral nutrition accumulation traits in maize grain (Lung'aho et al., 2011; Qin et al., 2012; Simic et al., 2012; Baxter et al., 2012), our approach is of great interests with highly novel originality. First, many mineral elements (Cu, Fe, K, Mg, Mn, P, and Zn) were here considered for QTL analysis, unlikely that a few elements, i.e. Fe and Zn, were investigated previously. Second, three mineral nutrition accumulation related traits (concentration, content and yield) were simultaneously considered while the previous studies were restricted to the concentration alone. Third, in this work four environments were used for phenotypic analysis, allowing to identify the stable (across environments) QTL for grain mineral related traits.<br><br>Our findings clearly revealed in maize grain the significant correlations between mineral concentration and content for each pair of elements, but not between nutrition concentration and seed mass traits (grain yield and seed weight). This points to the possibility to increase nutrient concentrations as a whole without affecting maize grain yield. We also identify the stable QTLs for mineral accumulation in maize grain from multiple environments that can help breeders to develop biofortification via MAS approaches.<br><br>We believe that these findings provide a fundamental progress in both field of plant nutrition and plant breeding. We therefore expect that this report will be frequently cited and substantially contribute to the high reputation of TAG.<br><br>Thank you for consideration of our manuscript.<br><br>Yours sincerely ,<br><br>Lixing Yuan |
| **Suggested Reviewers:** | Philp White, Ph.D<br>The James Hutton Institute<br>philip.white@hutton.ac.uk<br>He is engaged on projects addressing plant mineral nutrition |
| | Xianxun Xia, Ph.D<br>chinese academy agricultural sciences<br>xiaxianchun@caas.net.cn<br>He is an expert on QTL mapping for cereal (wheat and maize). |
| | Jan Schjoerring, Ph.D<br>University of Copenhagen<br>jks@plen.ku.dk<br>He is a famous professor on transporting of mineral elements in plant |

# Comprehensive phenotypic analysis and quantitative trait locus identification for seed mineral concentration, content, and yield in maize grains

Riliang Gu [1#], Fanjun Chen [1#], Bingran Liu [1], Xin Wang [1], Jianchao Liu [1], Pengcheng Li [1], Qingchun Pang [1], Jordon Pace [2], Thomas Lübberstedt [2], Guohua Mi [1] and Lixing Yuan [1*]

[1] *Department of Plant Nutrition, College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China*

[2] *Department of Agronomy, 1211 Agronomy Hall, Iowa State University, Ames, IA 50011, USA*

*# These authors contributed equally to this work*

** Corresponding author:* Lixing Yuan, Email: yuanlixing@cau.edu.cn; Phone: +86-10-62734424

**Author Contribution Statement:**

Conceived and designed the experiments: Lixing Yuan, Fanjun Chen and Riliang Gu. Analyzed the date: Riliang Gu, Fanjun Chen, Xin Wang and Jordon Pace. Performed field experiments: Fanjun Chen, Jianchao Liu, Pengcheng Li and Qingchun Pang. Performed lab measurement: Bingran Liu. Wrote the paper: Riliang Gu. Made the final revision: Lixing Yuan, Guohua Mi and Thomas Lübberstedt.

**Key Message:** Understanding the correlations of seven minerals for concentration, content and yield in maize grain, and exploring their genetic basis will help breeders to develop high grain quality maize.

**Conflict of Interest:** The authors declare that no conflict of interest exists.

**Abstract**

Biofortification by enhanced mineral density in maize grain through genetic improvement is one of the efficient ways to solve global mineral malnutrition, in which one key step is to detect the corresponding Quantitative Trait Loci (QTL). In this work, a maize recombinant inbred population (RIL) was grown to maturity in four field environments with two locations × two years. Phenotypic data of mineral nutrition concentration, content and yield were determined for grain copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), magnesium (Mg), potassium (K) and phosphorus (P). Analysis of variance (ANOVA) showed significant effects of genotype, location and year for all investigated traits. Location showed the highest effect for all mineral yields, and Zn and Cu content and concentration, while year had the strongest impact for Mn, K, and P content and concentration. Heritabilities ($h^2$) of different traits varied with higher $h^2$ (72-85%) for mineral concentration and content and lower (48-63%) for nutrient yields. Correlation coefficient analysis revealed significant positive correlations for grain concentration between several minerals. P had the closest correlations to other elements, while Cu had the lowest. When environments were analyzed individually, a total of 28, 25, and 12 QTL were identified for nutrient concentration, content and yield, respectively. Among these QTL, 8 QTL were consistent within traits across different environments. These stable QTL may be most promising for controlling mineral accumulation in maize grain. Co-localization of QTL for different traits was found for 12 chromosome regions, suggesting that common processes might contribute seed nutrient accumulation.

**Key words:** maize, nutrient elements, grain quality, quantitative trait locus.

## Introduction

The human body requires more than 22 mineral elements that can be supplied by an appropriate diet (Philip and Martin 2005). However, diets of human populations subsisting largely on cereals often lack Fe, Zn, Cu, Mg, and Mn, which are known to play an essential roles in physiological processes of the human body. Over three billion people in the world are affected by mineral nutrient malnutrition resulting in poor health and higher rates of mortality (Cakmak 2002). Many of those afflicted are dependent upon staple crops for their sustenance (Pfeiffer and McClaferty 2007). Among the staple crops, maize (*Zea mays* L.) is one of the most important crops, responsible for 40 percent of the world's cereal food production (Bouis and Welch 2010). Together with the fact that maize has the highest average yield per hectare, even a small increase in the nutritive value of maize would be important for human nutrition. Moreover, nutritional elements (e.g., Fe, Mn, Cu, and Zn) concentrations in maize grain are relatively low when compared to animal food products (Wang *et al.* 2003). Furthermore, the concentration even decreased in the past decades when exclusively grain yield but not quality was improved by breeders (Fan *et al.* 2008; Šimić *et al*. 2009; Anandan *et al.* 2011). Thus, since the concentration of these dietary minerals in maize grain are not sufficient to meet the dietary requirement of humans daily intake when these foods are consumed in typical amounts, improving the mineral concentration in maize grain is of great interest.

Traditional efforts on the improvement of mineral nutrition in crops were taken by the selection of germplasm with greater quantities of essential minerals, the breeding of mineral efficient crops that accumulate more minerals and enhancing bio-available minerals in edible portions. Some of these approaches had limited success, while others encountered problems that prevent their widespread acceptance. The amount of time and labor consumed for traditional efforts were the major blocks to meet a success. Quantitative trait locus (QTL) mapping is a powerful approach to study and manipulate complex traits important in agriculture (Abiola *et al*. 2003). A wide range of genetic variation for grain mineral amount has been revealed among maize accessions,

suggesting that mineral density traits are controlled by multiple loci across the genome (Šimic *et al*. 2012; Qin *et al*. 2012). Therefore, QTL analysis could greatly accelerate genetic modification of mineral amount in maize grain by marker-assisted selection (MAS) and even discovery of underlying candidate genes.

Concentration was widely applied for evaluating mineral amount in crop grain. Several studies have recently been published for rice, wheat, and soybean on identification of QTL for mineral concentration in grain (Stangoulis *et al.* 2007; Blair *et al.* 2009; Peleg *et al.* 2009; Tiwari *et al.* 2009; Chandel *et al.* 2011). In maize, QTL mapping of grain mineral concentration detected QTL distributed across the ten maize chromosomes; while localization of these QTL was inconsistent among different studies (Garcia-Oliveira *et al*. 2009; Zhou *et al*. 2010; Lung'aho *et al*. 2011; Qin *et al*. 2012; Šimic *et al*. 2012; Baxter *et al*. 2014). Qin *et al*. (2012) identified 31 QTL in a $F_{2:3}$ population for Fe and Zn concentration in two environments; three consistent QTL across different environments were detected in Bins 2.07, 7.04, and 9.07. Lung'aho *et al*. (2011) used B73×Mo17 (IBM population) to identify three Fe concentration QTL located in Bins 1.05, 3.04, and 9.00. Using an $F_4$ population, Šimic *et al*. (2012) identified 32 QTL for P, Fe, Zn, and Mg concentration using four environments and found a major QTL for micronutritional concentration located in Bin 3.05. The lack of common QTL identified from previous studies may result from the differences in mapping populations, genotypes and environments used in these studies. Therefore, more QTL analyses for these traits are necessary in order to detect more loci and ultimately identify consistent QTL for future fine mapping, MAS and map-based cloning.

Besides concentration, content per seed was considered to be important for evaluating mineral density in grain. A few studies indicated that the difference in mineral concentration between different individuals might simply represent a dilution effect due to grain size (Cakmak *et al*. 2000; Imtiaz *et al*. 2003); QTL analysis for both concentration and content enables to assess the potential colocalization of concentration and content loci for each mineral. Moreover, mineral element yield is important for evaluating the economic value for global

micronutrient malnutrition. In the present study, 218 recombinant-inbred lines (RIL) derived from a cross between inbred line Ye478 and Wu312, were used as a mapping population in order to map main-effect QTL for Fe, Ca, Zn, Mn, Mg, P, and Cu accumulation traits (concentration, content, and yield) in maize grain based on field measurements. The objectives were to 1) evaluate mineral elements concentrations, content and yield in the RIL population; 2) analyze their consistency across environments using two locations × two years; and 3) map QTL for element concentration, content, and yield in this population.

## Materials and methods

### Plant materials and field design

A population of 218 $F_8$ recombinant inbred lines (RILs) derived from the cross Ye478×Wu312 using single seed descent method was used in this study (Liu *et al*. 2011). RILs and their parents were grown in Beijing (BJ, 40°06′ N latitude, 116°11′ E longitude) and Gansu (GS, 38°37′ N latitude, 102°40′ E ) for two years (Table 1). Before planting, six soil samples were taken from the 0 - 30 cm soil layer for each environment, mixed, air-dried, and sieved to remove un-decomposed plant material, and used to measure the chemical properties (Table 1). Concentration of Fe, Mn, Zn, and Cu was determined by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES), while other element concentrations were failed to be extracted from soil samples by this method.

The experiment was arranged as split-plot design. RILs were planted in one-row plots with three replications. The rows were 4 m long containing 13 plants, with a plant density of 60,000 per hectare. The space between rows was 0.5 m. All fertilizers were applied before sowing, except nitrogen was supplied with two applications: 50% at sowing and 50% at the V6 (6 leave) stage. Rainfall during the growing season in Beijing ranged from 400 to 600 mm (Table S1), which was sufficient to avoid water deficit stress. Annual precipitation

in Gansu was only 100 to 300 mm, which was insufficient for maize growth. So, 500 mm irrigation water was additionally supplied. Other standard agronomic practices and need based plant protection measures were adopted uniformly to raise the crop.

**Phenotyping for quality traits**

The analysis methods used are almost identical to those described by Xue *et al*. (2014). At harvest, five plants were chosen from the middle of each row for trait evaluation. All panicles from these plants were harvested for evaluation of grain yield. Then 20 kernels were collected from the middle of each panicle, rapidly washed with deionized water and oven-dried at 70 °C to determine seed dry weight. For chemical element analysis, the collected kernels from each row were mixed and ground with a stainless steel grinder GENO-2000 (Spex, Pittsburg, CA, USA) and 0.5 g powder was digested with $HNO_3$-$H_2O_2$ in a microwave accelerated reaction system (CEM, Matthews, NC, USA). The concentrations of chemical elements in the digested solutions were determined by inductively coupled plasma atomic emission spectroscopy (ICP-OES, Perkin-Elmer, USA).

**Data analysis**

The chemical element content (g/seed) was calculated as: element concentration × kernel weight; and yield (g/ha): element concentration × grain yield. Since the experiments were set up as randomized complete blocks (RCBD) with three replicates, the Least Square Mean (LSMEAN) of the replicates was used for QTL analysis of the measured parameters from each environment. Then these data were analyzed by analysis of variance (ANOVA) using the SAS statistics system. For each phenotypic value the following a linear model was used:

$$Y_{ijkr} = \mu + G_i + L_j + Y_k + G \times L_{ij} + G \times Y_{ik} + L \times Y_{jk} + G \times L \times Y_{ijk} + e_{ijkr},$$

where observation $Y_{ijkl}$ is the plot based phenotype as sum of the mean ($\mu$), the genetic effect (G) of the $I_{th}$ line,

the effect of the $J_{th}$ location (L), the effect of the $K_{th}$ Year (Y), and their respective interactions $G \times L_{ij} + G \times Y_{ik} + L \times Y_{jk} + G \times L \times Y_{ijk}$ and the error $e_{ijkr}$. The PROC MIXED procedure (SAS) was used to estimate LSMEAN values, which were used to analyze trait Pearson correlations (Pillen *et al*. 2003).

Trait heritabilities were calculated from an ANOVA fitting effects of genotype (G), environment (E, locations and year were combined into 4 environments), and G × E interactions, as

$$h^2 = \sigma^2_G / (\sigma^2_G + \sigma^2_{GE} / n + \sigma^2_E/nr) \times 100$$

where $h^2$ is broad sense heritability, $\sigma^2_G$ is genotypic variance, $\sigma^2_{GE}$ is genotype × environment variance, $\sigma^2_E$ is error variance, n is number of environments and r is the number of replications (Nyquist 1991).

Using 184 polymorphic markers, a genetic linkage map was constructed for this RIL population with a total length of 2084 cM and an average interval of 11.3 cM in our previous work (Liu *et al.* 2011). QTL were detected by composite interval mapping (Zeng 1994) using Windows QTL Cartographer version 2.5 (Model 6) (Wang *et al.* 2012). The threshold LOD value was determined with 1000 permutations at P=0.05 level (Churchill and Doerge 1994). Locus position was assigned as the point of maximal LOD score. Loci detected within the average interval (11.3 cM) in different environments were considered to be the same locus for each trait.

## Results

### Grain mineral concentration, content and yield in parents and RILs

All RILs were grown to maturity in four environments at Gansu (GS) and Beijing (BJ) locations in 2009 and 2010: GS09, GS10, BJ09 and BJ10 (Table 1). Grain mineral accumulation traits including concentration, content, and yield were investigated for four different micronutrients (copper (Cu), iron (Fe), manganese (Mn) and zink (Zn)), and three different macronutrients (magnesium (Mg), potassium (K) and phosphorus (P)). Grain yield (GY) and seed weight (SW) were also evaluated. Between the two parents, line Ye478 had significantly higher grain

yield and seed weight compared to Wu312 across all environments (Table 2). For most mineral elements, the concentration showed similar levels in both parents, while the content and yield were higher in Ye478 with a higher SW and GY. However, Fe concentration was significantly higher in Ye478 across all environments, except in BJ09, and the P and Zn concentrations were significantly higher in Wu312 in BJ09.

Among RILs the values of all investigated traits segregated continuously and approximately fit normal distributions with absolute values of both skewness and kurtosis being less than 1.0. All traits showed transgressive segregation in both directions (lines with lower values than the lowest parent or higher values than the highest parent) (Supplementary Fig. S1). These results suggest presence of multiple genes controlling the investigated traits. Average SW was similar across environments, while GY and some mineral elements markedly varied among different environments (Table 3). Grain yield in GS was similar in both years and higher than in BJ. At BJ, the lowest GY was found in 2010, likely a result from drought stress at maize silking stage (approx. 25% less than average monthly rainfall in July 2010: Table S1). Concentration and content of most elements (except Cu and Zn) were similar across environments with the exception of BJ where higher value was found likely due to the concentrating effect by its lower GY (Table 3). Nutrient yields were higher for all chemical elements in GS than in BJ.

**Phenotypic variation and heritability for mineral concentration, content, and yield**

The effect of genotype was significant for all traits ($\alpha = 0.05$) (Table 4). Effects of environment (year and location) and genotype × environment were also significant, suggesting the presence of strong environmental effects on nutrition accumulation in maize grain across these two locations and years. Within environmental effects, location had a stronger effect than year for GY, SW, and all mineral yields. Cu and Zn had higher location effects for both concentration and content, while Mn, K, and P showed stronger year effects. Fe and Mg

had higher year effect for their concentration, but lower for content.

The order of accumulation levels of the investigated elements in maize grain were as follows: K>P>Mg>Fe>Zn>Mn>Cu (Table 3). Despite the lower level of accumulation, Cu and Mn had higher coefficients of variation (CV%) values (18.2-28.8%, 22.3-31.9%, 33.4-55.3%) for concentration, content and yield, respectively (Table 3), indicating that nutrition elements with lower levels had greater genetic variation within the population. The heritability ($h^2$) of different traits varied from 48 to 85% with an average of 70% (Table 4). Element concentration and content had similar $h^2$ at higher levels of 72 to 85%, while element yields had lower levels of $h^2$ ranging from 48 to 63%.

**Correlations of mineral concentration, content, and yield**

We found significant positive correlations for grain concentration between several minerals (Table 5). Some of the minerals were highly correlated while many of them had weak positive correlations. Among these elements, P had the closest correlation to Mg concentration ($r=0.65$, $p<0.01$), and medium correlation to Fe, K, Mn, and Zn concentrations ($r=0.39$-$0.48$, $p<0.01$). All element concentrations showed no significant correlation to grain yield or seed weight. Close correlations (significantly positive) were observed for nutrient content and element yield between each two mineral elements ($r=0.33$-$0.96$, $p<0.01$). Seed weight and grain yield showed positive correlations to all element contents ($r=0.29$-$0.86$, $p<0.01$) and element yields (0.69-0.89, $p<0.01$), respectively.

**Identification of grain yield, seed weight and seed mineral element QTLs**

LOD threshold values were 2.9–3.3 for different traits (Supplementary Table S2). Among the 23 investigated traits, we identified 74 QTL in total for 21 traits across four environments (Fig. 1 & Supplementary

Table S3). No QTL was found for Cu concentration and Fe yield. The percentage of explained variance for QTL varied from 5.84 to 38.14%. Two QTL, *qZnCT1* and *qMgCC10*, explained more than 30% of the phenotypic variance with 31.4% and 38.1%, respectively. Another four QTL explained between 20% and 30% phenotypic variance (Supplementary Table S3). Five and six QTL were identified for grain yield and seed weight, respectively. For the mineral traits, 24, 27, and 12 QTL were identified for the mineral concentrations, content and yield, respectively.

QTL repeatedly detected across the different environments were considered as stable QTL (sQTL). About 77% QTL (57 out of the 74 QTL) were only detected in a single environment, while other 17 QTL were detected in at least two environments. As a consequence, 8 sQTL were identified based on these 17 QTL with one detected in three environment and 7 in two environments (Table 6).

For grain yield, no sQTL was identified. Three of the five QTL detected from BJ10 with a total of 39.6% phenotypic variance explained, and all yield-increasing alleles originated from parental line Ye478 (Supplementary Table S3). A sQTL (*sQTL3.1*) was observed in Bin3.08/3.09 for SW in three environments (GS09, GS10, and BJ09) explaining phenotypic variance of 20.8%, 16.8%, and 27.3%, respectively (Table 6).

Among 13 Zn-related QTL, two sQTL were detected for Zn content and concentration located in in Bin 4.07 and 5.03/5.04, respectively (Table 6). These sQTL were detected in two environments explaining 7.0-12.2% of the total variance. In addition, co-localization of QTL for Zn concentration (*qZnCC5.4*) and yield (*ZnYD5*) was observed on chromosome regions of Bin 5.06 (Fig. 1 & Table 7).

Six QTL were identified for Mn concentration with similar explained variance ranging from 10.8% to 15.3%, and assigned to 3 sQTL groups to Bin 1.04/1.05, 2.03/2.04, and 4.05/4.06 (Table 6). In addition, two QTL were identified for Mn content, and one of them *(qMnCT1*) was also localized in Bin1.04/1.05.

Among the four QTL for K content, one sQTL in Bin 3.08/3.09 was detected in both GS10 and BJ09

environments which explained phenotypic variance of 6.2% and 27.8%, respectively (Table 6 & Supplementary Table S3). Six QTL were observed for Mg concentration, including one sQTL (sQTL3.1) in Bin3.04 observed at two environments (Table 6). Three QTL were observed for Mg content and one of them (*qMgCT3*) in Bin3.04 coincided with sQTL3.1 for Mg concentration (Supplementary Table S3). Thus, the chromosome region Bin3.04 might be a hot-spot region for controlling Mg accumulation in maize grain.

Seven, six, and four QTL were identified for Cu, Fe, and P related traits across the four environments, respectively (Supplementary Table S3). The explained variances for Cu, Fe, and P related QTL were relative small ranging from 6.1% to 16.1%. In addition, no sQTL was found for these elements. These results indicate that Cu, Fe, and P accumulation in maize grain are controlled by minor effect QTL.

## Discussion

### Genetic and phenotypic relationship between nutrient element concentration, content, and yield in maize grain

Enhancement of mineral nutrient density (biofortification) in crop grain is one of the efficient ways to solve global nutrient malnutrition, in which one key step is to detect favorable QTL/genes/alleles. Several studies on QTL mapping for maize grain minerals have detected QTL distributed across the ten chromosomes, and focused primarily on the most important elements, Fe and Zn (Zhou *et al*. 2010; Lung'aho *et al*. 2011; Šimic *et al*. 2012). Beside Fe and Zn, QTL for Mg and P concentration, as well as Fe/P, Zn/P, and Mg/P ratio in maize grain were analyzed to detect three P related QTL co-localizing on chromosome 3 (Šimic *et al*. 2012). Genetic study on other elements (e.g. Cu, K and Mn) in maize grain was recently reported through single-kernel ionomic profile (Baxter *et al*. 2014). However, the phenotypic and genetic correlations among mineral elements have not been addressed in the previous works. Here we conducted QTL analysis for seven elements (Cu, Fe, K, Mg, Mn, P,

and Zn) as well as traits of grain yield and seed weight. No significant correlations were found for any element concentration with grain yield or seed weight (Table 5), suggesting that increasing nutrient concentration in maize grain may have no effect on grain yield. In addition, excluding Mn and Mg, no QTL co-location was observed between nutrition concentration and either grain yield or seed weight, further indicating that no or low genetic correlation between nutrition concentration and seed mass traits in maize. Conversely, a significant correlation was found between each pair of element (except Cu) concentration (Table 5). Taken together, these results indicate that it is possible to increase several nutrient concentrations as a whole without affecting grain yield in maize.

In contrast to the previous studies focused on grain-concentration, we analyzed three mineral nutrient accumulation traits: concentration, content, and yield. QTL analysis for both concentration and content helped to identify co-localized concentration and content loci for each mineral. Four chromosome regions were identified containing both concentration and content QTL (Table 7). QTL for Mg concentration (*qMgCC3.1* and *qMgCC6*) and content (*qMgCT3* and *qMgCT6*) were found in Bin3.04 and Bin6.07/6.08, respectively. Co-localization of concentration and content QTL were also found for Mn and Zn in Bin1.04/1.05 and Bin5.05, respectively. Together with the fact that the favorable alleles for these four pairs of concentration and content QTL came from the same parent (Supplementary Table S3), explains genetic correlations between nutrient content and concentration in maize grain.

**Environmental influences to mineral nutrition accumulation in maize grain**

Nutrition accumulation in maize grain is a complex trait affected by a number of factors, including genotype, environmental conditions and their interactions (House *et al*. 1999; Baxter *et al*. 2012). Plant breeders usually evaluate their genetic materials in several environments to minimize the environmental effects and identify

stable alleles (and genotypes). In this work, four environments with two locations × two years were applied to test the environmental effects on mineral nutrient accumulation in maize grain. The ANOVA showed significant ($P<0.01$) effects for both year and location (Table 4) for all investigated mineral nutrient concentrations, contents and yields, in agreement with previous studies (Qin *et al*. 2012) .

Soil conditions and regional climatic factors considerably impact the environmental performance for crop production (Pacini *et al.* 2003). Climatic conditions during maize growing season differed in these four experimental environments (Table S1). BJ10 was extremely dry and total rainfall in the growing period achieved only 25% of the long term average value. The severe drought condition in BJ10 resulted in approximately 70% yield loss compared to the average yield from the other three environments (Table 3). Higher values for all tested mineral elements was found in BJ10, which may be attributed to a concentration effect, i.e., reverse dilution effect due to lower grain yield and lower carbohydrate accumulation. However, the genetic features for the tested mineral accumulation traits in BJ10 were similar to other environments, these included: 1) the variance coefficient for each element's concentration, content, and yield in BJ10 was similar to the other three environments (Table 3); 2) the number of QTL (15) detected at BJ10 was similar to other environments (the average was 16) (Supplementary Table S3); 3) eight sQTL repeatedly detected for 17 QTL across environments in a similar number from each environment with 2 from BJ10 (the average was 4) (Table 6); 4) Twelve co-localized QTL across elements (for at least two elements) were identified from 50 QTL in a similar number in each environment with 12 from BJ10 (the average was 13) (Table 7). All these similar features suggested that BJ10 environment was suitable for QTL analysis in this work and could be combined with the other three environments as a comprehensive experiment, despite of drought stress.

Soil properties for the two locations GS and BJ showed differences in mineral availabilities. Available Zn in BJ was higher than that in GS. Consistently, maize grain Zn density in BJ was also found to be higher (Table 3).

In addition ANOVA analysis found higher location effect for both Zn concentration and content, but not for other elements (except Cu) (Table 4), further suggesting that soil Zn availability plays a critical role for its accumulation in maize grain. These results are consistent with other studies (Sharma and Bapat 2000; Chahal *et al*. 2005). These results suggest that Zn accumulation in maize grain can be significantly improved by increasing soil Zn availability, e.g., through Zn fertilizer. Indeed, a recent work has found that Zn deficiency can be corrected by the application of Zn fertilizer to soils (Kumar *et al*. 2014).

For other elements, e.g., Cu, Fe, and Mn, the effects of soil element availability for grain element accumulation are not resolved. Kumar *et al.* (2009) reported that Cu concentrations in leaves, grain, and straw increased significantly with an increase in the level of applied Cu in soil. Antunović *et al.* (2003) found that the mineral concentrations of Mn and Fe in maize grain were not in connection to the status of the corresponding elements in the experimental environments. It was presumed that interaction with other elements (e.g., Ca or Mg) could be responsible for this inconsistence for Cu, Fe, and Mn. We found that soil available Cu, Fe, and Mn were higher in GS location (Table 1). However, grain nutrition element accumulation in GS was similar to that in BJ (Table 3), except that BJ10 had higher element concentrations due to drought stress. Thus, our results suggest little effect of soil available Cu, Fe, and Mn on their accumulation in maize grain.

**Important QTL clusters for mineral nutrition accumulation in maize grain**

Limited information is available about the genetic control and molecular mechanism contributing to high accumulation of mineral elements in maize grain. QTL analysis provides a powerful tool to point out chromosomal locations of genes suitable for breeding programs. However, identified QTL for maize grain mineral accumulation traits have been inconsistent in previous studies (Garcia-Oliveira *et al*. 2009; Zhou *et al.* 2010;Lung'aho *et al*. 2011; Šimic *et al*. 2012; Baxter *et al*. 2014). In this work we conducted a QTL analysis in

four environments and found several important chromosome regions which contained stable (across different environments) QTL within a grain mineral trait or contained co-localized QTL cross different traits (Fig. 1; Table 6 & 7). In general, 52 QTL (70%) out of the 74 detected QTL fell into 12 QTL clusters in which 3-6 QTL were grouped for different mineral traits or from different environments within a trait.

In chromosome region Bin5.05, five QTL were identified with one for seed weight and four for nutrition content of K, P, Zn, and Mg (Fig. 1 & Table 7). Interestingly, all five QTL were identified in the GS10 environment. In another genomic region, Bin6.07/6.08, five QTL were detected at GS10, with two for Mg related traits and four for SW and K, P, and Zn content. Zhou *et al*. (2010) identified two QTL located in this region for Fe and Zn concentration from two different populations but in the same environment, which suggests Bin6.07/6.08 and 5.05 might contain environment specific regions for controlling seed mineral nutrition accumulation.

Three out of the five QTL identified in Bin3.08/3.09 were SW QTL, which represent a sQTL (sQTL3.1) that was repeatedly detected in three environments (Table 6). In this region, two QTL for K content were also clustered. Some important QTL for mineral element accumulation were also identified in this region in previous studies. Šimic *et al.* (2012) found a QTL for P concentration by using a $F_4$ population of B84 × Os6-2 and Lung'aho *et al.* (2012) found a Fe bioavailability QTL from B73 × Mo17 RIL (IBM) population. These results suggest an important chromosome region of Bin3.08/3.09 for both seed mineral accumulation and seed physiological traits.

In chromosome Bin1.07 genomic region, we identified several mineral nutrition yield QTL (Supplementary Table S3), including *qMnYD1*, *qZnYD1* and *qMgYD1*. These QTL explained 17.8-19.04% of the phenotypic variance. Interestingly, a QTL for grain yield was also localized in this region (Supplementary Table S3), which could explain the major functions of this QTL region in controlling grain yield and nutrition yield.

In the region Bin 4.05/4.06, a sQTL (sQTL4.1) contained 2 QTL for Mn concentration was identified within a cluster containing other mineral related QTLs (Table 6). The existence of QTL affecting Mn concentration was also reported in the same region in other maize populations (Zhou *et al.* 2010). In another chromosomal region Bin1.04/1.05, three QTL for Mn related traits were identified (Supplementary Table S3). These results suggest that Bin4.05/4.06 and 1.04/1.05 might be the major chromosome loci response for Mn accumulation in maize grain.

In other clusters, QTL for several different mineral elements were clustered (Table 7). For example, in the region Bin4.07, a sQTL (sQTL4.2) for Zn content from BJ09 and GS09 environments was identified within a cluster containing four other element content QTL for Cu, Mn, P and Fe. In this region, Šimic *et al.* (2012) detected a QTL for Fe/P ratio. In Bin3.04, a sQTL (sQTL3.1) for Mg concentration was identified within a cluster containing *qMgCT3*, *qCuCT3-1*, *qPCC3*, and *qKCC3*. Interestingly, Lung'aho *et al.* (2011) identified an important QTL for Fe bioavailability from a RIL population; and Qin *et al.* (2012) identified a QTL for Zn concentration via joint QTL mapping in two environments in the same region. These observations indicate that these particular genomic regions seem to be important for seed mineral element accumulation in maize grain. QTL co-localization across mineral elements further supported the common ideas of some loose linkage among mineral elements by their functionally co-adaption, or relation to unknown factor(s) with pleiotropic effect, such as the ionic forms of Fe, Zn, Cu, Mn, and Mg acted as co-factors in many enzymes e.g. enzymes in photosynthesis and respiration processes (Marschner 1995; Mengel *et al.* 2001).

**Acknowledgements**

This study was supported by the Ministry of Science and Technology of China (2012AA100306, 2011CB100305); National Natural Science Foundation of China (31121062); the Ministry of Agriculture of

China (2014ZX08003-005) and Danish Strategic Research Council (NUTRIEFFICIENT 10-093498).

**Reference**

Abiola O, Angel JM, Avner P, Bachmanov AA, Belknap JK *et al.* (2003) The nature and identification of quantitative trait loci: a community's view. Nat Rev Genet 4: 911–916

Anandan A, Rajiv G, Eswaran R, Prakash M (2011) Genotypic variation and relationships between quality traits and trace elements in traditional and improved rice (Oryza sativa L.) genotypes. J Food Sci 76:122-130

Antunović M, Kovačević V, Rastija M, Zdunić Z (2003) Influences of soil and genotypes on micronutrient status in maize plants. Agriculture 9: 9-14.

Baxter I, Dilkes BP (2012) Elemental profiles reflect plant adaptations to the environment. Science 336: 1661–1663.

Baxter IR, Ziegler G, Lahner B, Mickelbart MV, Foley R *et al.* (2014) Single-Kernel Ionomic Profiles Are Highly Heritable Indicators of Genetic and Environmental Influences on Elemental Accumulation in Maize Grain (Zea mays). PLoS ONE 9: e87628.

Blair MW, Astudillo C, Grusak MA, Graham R, Beebe SE (2009) Inheritance of seed iron and zinc concentrations in common bean (Phaseolus vulgaris L.). Mol Breeding 23:197–207.

Bouis HE, Welch RM (2010) Biofortification—a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. Crop Sci 50: S20-32.

Cakmak I (2002) Plant nutrition research: priorities to meet human needs for food in sustainable ways. Plant Soil 247: 3-24.

Cakmak I, Pfeiffer WH, Mcclafferty B (2010) Biofortification of durum wheat with zinc and iron. Cereal Chem 87: 10–20.

Chahal DS, Sharma BD, Singh PK (2005) Distribution of forms of zinc and their association with soil properties and uptake in different soil orders in semi-arid soils of Punjab, India. Commun Soil Sci Plant Anal 36: 2857–2874.

Chandel G, Samuel P, Dubey M, Meena R (2011) In silico expression analysis of QTL specific candidate genes for grain micronutrient (Fe/Zn) content using ESTs and MPSS signature analysis in rice (Oryza sativa L.). J Plant Genet Transgenics 1:11–22

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138: 963–971.

Fan MS, Zhao FJ, Fairweather-Taitc SJ *et al*. (2008) Evidence of decreasing mineral density in wheat grain over the last 160 years. Journal of Trace Elements in Medicine and Biology 22: 315-324

Garcia-Oliveira AL, Tan LB, Fu YC, Sun CQ (2009) Genetic identification of quantitative trait loci for contents of mineral nutrients in rice grain. J Integr Plant Biol 51: 84–92.

House WA (1999) Trace element bioavailability as exampled by iron and zinc. Field Crops Res 60: 115-141.

Imtiaz M, Alloway BJ, Shah KH, Siddiqui SH, Memon MY, Aslam M, Khan P (2003) Zinc nutrition of wheat: Growth and zinc uptake. Asian J Plant Sci 2:152-155.

Kumar R, Bohra JS (2014) Effect of NPKS and Zn application on growth, yield, economics and quality of baby corn. Arch Agron Soil Sci 60:1193-1206.

Kumar R, Mehrotra NK, Nautiyal BD, Kumar P, Singh PK (2009). Effect of copper on growth, yield and concentration of Fe, Mn, Zn and Cu in wheat plants (Triticum aestivum L.). J Environ Biol 30: 485-488.

Liu J, Cai H, Chu Q, Chen X, Chen F, Yuan L, Mi G, Zhang F (2011) Genetic analysis of vertical root pulling resistance (VRPR) in maize using two genetic populations. Mol Breeding 28: 463–474.

Lung'aho MG, Mwaniki AM, Szalma SJ, Hart JJ, Rutzke MA, Kochian LV, Glahn RP, Hoekenga OA (2011)

Genetic and physiological analysis of iron biofortification in maize kernels. PLoS One. 6:e20429.

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. London: Academic Press.

Mengel K, Kirk EA, Kosegarten H, Appel T (2001) Principles of plant nutrition. Dordrecht: Kluwer Academic press.

Nyquist WE (1991) Estimation of heritability and prediction of selection response in plant populations. Crit Rev Plant Sci 10:235–322

Pacini C, Wossink A, Giesen G, Vazzana C, Huirne R (2003) Evaluation of sustainability of organic, integrated and conventional faming systems: a farm and field-scale analysis Agriculture. Ecosys Environ 95:273–288

Peleg Z., Cakmak I, Ozturk L, Yazici A, Jun Y, Budak H, Korol AB, Fahima T, Saranga Y (2009) Quantitative trait loci conferring grain mineral nutrient concentrations in durum wheat× wild emmer wheat RIL population. Theor Appl Genet 119: 353–369.

Pfeiffer WH, McClafferty B (2007) HarvestPlus: Breeding crops for better nutrition. Crop Sci 47: S88-105.

Pillen K, Zacharias A, Léon J (2003) Advanced backcross QTL analysis in barley (Hordeum vulgare L.) Theor Appl Genet 107:340–352.

Philip JW, Martin RB (2005) Biofortifying crops with essential mineral elements. Trends Plant Sci 10: 586-593.

Qin HN, Cai LY, Liu ZZ, Wang GQ, Wang JG, Guo Y, Wang H (2012) Identification of QTL for zinc and iron concentration in maize kernel and cob. Euphytica 187: 345–358.

Sharma BL, Bapat PN (2000) Levels of micronutrient cations in various parts of wheat and influenced by zinc and phosphorus application. J Indian Soc Soil Sci 48: 130 – 134.

Šimic D, Drinic SM, Zdunic Z, Jambrovic A, Ledencan T, Brkic J, Brkic A, Brkic I (2012) Quantitative trait loci for biofortifica tion traits in maize grain. J Hered 103: 47-54.

Šimic D, Sudar R, Ledencan T, Jambrovic A, Zdunic Z, Brkic I, Kovacevic V (2009) Genetic variation of

bioavailable iron and zinc in grain of a maize population. J Cereal Sci 50: 392-397

Stangoulis JCR, Huynh B, Welch RM, Choi E, Graham RD (2007) Quantitative trait loci for phytate in rice grain and their relationship with grain micronutrient content. Euphytica 154:289–294.

Tiwari VK, Rawat N, Chhuneja P, Neelam K, Aggarwal R, Randhawa GS, Dhaliwal HS, Keller B, Singh K (2009) Mapping of quantitative trait loci for grain iron and zinc concentration in diploid a genome wheat. J Hered 100:771–776.

Wang S, Basten CJ, Zeng ZB (2012) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC

Wang TL, Domoney C, Hedley CL, Casey R, Grusak MA (2003) Can we improve the nutritional quality of legume seeds? Plant Phys 131:886–891

Xue Y, Yue S, Zhang W, Liu D, Cui Z *et al*. (2014) Zinc, Iron, Manganese and Copper Uptake Requirement in Response to Nitrogen Supply and the Increased Grain Yield of Summer Maize. PLoS ONE 9: e93895.

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468.

Zhou JF, Huang YQ, Liu ZZ, Chen JT, Zhu LY, Song ZQ, Zhao YF (2010) Genetic analysis and QTL mapping of zinc, iron, copper and manganese contents in maize seed. J Plant Genet Resources 11: 593–595.

**Figure legends**

**Fig. 1.** Chromosome localization of QTL for grain yield, seed weight, and seed mineral element concentration, content and yield in maize detected in Ye478 X Wu312 RIL populations.

Each QTL to the left of the linkage map are above the LOD threshold for composite interval mapping (CIM) analysis. QTL are marked by its environment name with different sharp for different traits. ※, #,▲,★, ■, ◢, ●, ▼ and ◆ represented grain yield (GY), seed weight (SW) and iron (Fe), manganese (Mn), zink (Zn), copper (Cu), potassium (K), phosphorus (P) and magnesium (Mg) traits, respectively. Red, blue and yellow letters represents seed mineral concentration, content and yield, respectively. Chromosome Bins with yellow background represented important regions for controlling seed mineral traits with several QTLs co-localized here.

**Table legends**

**Table 1.** Soil environment and fertilizer supplement for plant growth at fields of Gansu (GS) and Beijing (BJ) in 2009 and 2010.

**Table 2**. Parent performance of mineral nutrient concentration, content and yield as well as seed weight (SW) and grain yield (GY) in maize inbred lines Ye478 and Wu312 under four environments.

**Table 3**. Seed mineral nutrient concentration (CC), content (CT) and yield (YD) as well as seed weight (SW) and grain yiled (GY) in the RIL population grown in four environments.

**Table 4.** Analysis of variances (ANOVA) and heritability ($h^2$) on seed mineral nutrition concentration, content and yield as well as grain yield (GY) and seed weight (SW) in the RIL population grown in four environments. .

**Table 5.** Correlation coefficients (r) between each two traits for grain yield (GY), seed weight (SW) and seed nutrition concentration, content and yield using LSMEAN values across four environments.

**Table 6.** Summary of stable QTL (sQTL) for all investigated traits across all the environments.

**Table 7.** Summary of chromosome clusters for all investigated traits across four environments.

**Supplementary materials**

**Supplementary Fig. S1.** Population distributions for grain yield, seed weight and seed nutrient (Cu, Fe, Mn, Zn, K, Mg and P) concentration, content and yield in the Ye478 × Wu312 recombinant inbred lines grown in four environments GS09, GS10, BJ09 and BJ10.

**Supplementary Table S1**. Monthly precipitation (mm) and 18 year-average (from 1996 to 2003) in Beijing location.

**Supplementary Table S2.** LOD threshold value for grain yield (GY), seed weight (SW) and seed nutrient concentration, content and yield.

**Supplementary Table S3.** QTLs detected for grain yield, seed weight and seed nutrient concentration, content and yield in four environments GS09, GS10, BJ09 and BJ10.

**Table 1.** Soil environment and fertilizer supplement for plant growth at fields of Gansu (GS) and Beijing (BJ) in 2009 and 2010.

| Environ-ment | Soil [a] | | | | | | | | Fertilizer | | | Plant density (plant/ha) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Organic matter (g/kg) | Total nitrogen (mg/kg) | Available phosphorus [b] (mg/kg) | Available potassium [c] (mg/kg) | Cu (mg/kg) | Fe (mg/kg) | Mn (mg/kg) | Zn (mg/kg) | N (kg/ha) | $P_2O_5$ (kg/ha) | $K_2O$ (kg/ha) | |
| GS09 | 19.3 | 1.24 | 24.5 | 173.8 | 1.98 | 11.9 | 17.31 | 1.01 | 180 | 120 | 80 | 60000 |
| GS10 | - | - | - | - | - | - | - | - | 180 | 120 | 80 | 60000 |
| BJ09 | 15.8 | 0.83 | 26.7 | 103.8 | 1.42 | 8.45 | 9.05 | 1.4 | 180 | 120 | 80 | 60000 |
| BJ10 | - | - | - | - | - | - | - | - | 180 | 120 | 80 | 60000 |

[a] Nutrient concentration in soil were measured before sowing in 2009,

[b] Available phosphorus was indicated by Olsen-P,

[c] Available potassium was indicated by exchangeable-K.

**Table 2**. Parent performance of mineral nutrient concentration, content and yield as well as seed weight (SW) and grain yield (GY) in maize inbred lines Ye478 and Wu312 under four environments.

| Trait | Environment | Concentration [a] | | Content [a] | | Yield [a] | |
|---|---|---|---|---|---|---|---|
| | | 478 | Wu312 | 478 | Wu312 | 478 [b] | Wu312 |
| GY (t/ha) | GS09 | | | | | 5.39 a | 4.67 b |
| | GS10 | | | | | 4.73 a | 3.83 b |
| | BJ09 | | | | | 4.39 a | 2.65 b |
| | BJ10 | | | | | 1.43 a | 1.32 a |
| SW (g/seed) | GS09 | | | | | 0.28 a | 0.23 a |
| | GS10 | | | | | 0.31 a | 0.24 b |
| | BJ09 | | | | | 0.33 a | 0.21 b |
| | BJ10 | | | | | 0.30 a | 0.19 b |
| Cu | GS09 | 1.47 a | 1.42 a | 0.40 a | 0.32 a | 7.97 a | 6.66 a |
| | GS10 | 1.36 a | 1.43 a | 0.42 a | 0.35 b | 6.47 a | 5.49 a |
| | BJ09 | 1.98 a | 2.21 a | 0.66 a | 0.47 b | 8.71 a | 5.86 b |
| | BJ10 | 1.53 a | 1.70 a | 0.45 a | 0.40 a | 2.16 a | 2.25 a |
| Fe | GS09 | 19.28 a | 12.97 b | 5.33 a | 2.95 b | 103.83 a | 60.81 a |
| | GS10 | 18.84 a | 13.66 b | 5.81 a | 3.31 b | 89.35 a | 52.30 b |
| | BJ09 | 16.87 a | 17.04 a | 5.62 a | 3.60 b | 74.36 a | 45.23 b |
| | BJ10 | 21.24 a | 17.53 b | 6.32 a | 3.37 b | 30.32 a | 19.16 b |
| Mn | GS09 | 4.97 a | 4.25 a | 1.24 a | 0.96 a | 24.25 a | 19.87 b |
| | GS10 | 4.31 a | 4.33 a | 1.33 a | 1.05 b | 20.42 a | 16.58 b |
| | BJ09 | 4.22 a | 5.65 b | 1.41 a | 1.19 b | 18.58 a | 15.01 a |
| | BJ10 | 5.34 a | 5.79 a | 1.59 a | 1.25 b | 7.64 a | 7.68 a |
| Zn | GS09 | 11.91 a | 12.84 a | 3.30 a | 2.92 a | 64.11 a | 60.22 a |
| | GS10 | 11.14 a | 13.33 a | 3.46 a | 3.23 a | 52.92 a | 50.99 a |
| | BJ09 | 14.94 a | 20.64 b | 4.98 a | 4.35 b | 65.69 a | 54.82 a |
| | BJ10 | 18.74 a | 20.91 a | 5.57 a | 4.69 a | 26.75 a | 27.69 a |
| K | GS09 | 3.52 a | 3.22 a | 0.98 a | 0.73 a | 19.02 a | 15.08 b |
| | GS10 | 2.90 a | 3.15 a | 0.90 a | 0.76 b | 13.74 a | 12.05 b |
| | BJ09 | 2.98 a | 3.61 b | 0.99 a | 0.76 b | 13.09 a | 9.57 b |
| | BJ10 | 3.82 a | 3.74 a | 1.14 a | 0.78 b | 5.47 a | 4.95 a |
| Mg | GS09 | 1.11 a | 0.97 b | 0.30 a | 0.22 a | 5.96 a | 4.52 b |
| | GS10 | 1.03 a | 0.93 a | 0.31 a | 0.23 b | 4.86 a | 3.57 b |
| | BJ09 | 0.96 a | 1.03 a | 0.32 a | 0.22 b | 4.21 a | 2.74 b |
| | BJ10 | 1.21 a | 1.04 a | 0.36 a | 0.22 b | 1.73 a | 1.37 a |
| P | GS09 | 2.46 a | 2.74 a | 0.68 a | 0.62 a | 13.22 a | 12.80 a |
| | GS10 | 2.36 a | 2.42 a | 0.73 a | 0.59 b | 11.20 a | 9.27 b |
| | BJ09 | 2.53 a | 3.42 b | 0.84 a | 0.72 b | 11.09 a | 11.09 a |
| | BJ10 | 3.27 a | 3.29 a | 0.97 a | 0.70 b | 4.68 a | 4.36 a |

[a] Mineral nutrient concentration, content and yield were expressed as mg/kg, mg/seed and g/ha for micronutrients (Cu, Fe, Mn, and Z), and g/kg, g/seed and kg/ha for macronutrients (K, Mg, and P), respectively.

[b] Significant differences between two parents in each environment were indicated by different letters ($P<0.05$).

**Table 3**. Seed mineral nutrient concentration (CC), content (CT) and yield (YD) as well as seed weight (SW) and grain yiled (GY) in the RIL population grown in four environments.

| Trait | Environment | Concentration [a] | | | Content [a] | | | Yield [a] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Range | cv (%) | Mean | Range | cv (%) | Mean | Range | cv (%) |
| GY (t/ha) | GS09 | | | | | | | 4.08 | 1.50-8.84 | 28.85 |
| | GS10 | | | | | | | 4.23 | 0.78-8.72 | 29.91 |
| | BJ09 | | | | | | | 2.86 | 0.70-7.06 | 41.53 |
| | BJ10 | | | | | | | 1.56 | 0.23-4.31 | 54.33 |
| SW (g/seed) | GS09 | | | | | | | 0.27 | 0.13-0.40 | 19.01 |
| | GS10 | | | | | | | 0.27 | 0.15-0.42 | 16.16 |
| | BJ09 | | | | | | | 0.26 | 0.13-0.39 | 18.77 |
| | BJ10 | | | | | | | 0.25 | 0.12-0.35 | 15.8 |
| Cu | GS09 | 1.34 | 0.75~2.40 | 27.55 | 0.36 | 0.15~0.75 | 31.92 | 5.42 | 1.94~12.17 | 35.26 |
| | GS10 | 1.45 | 0.70~2.70 | 28.84 | 0.39 | 0.19~0.77 | 29.38 | 6.23 | 1.64~15.29 | 39.87 |
| | BJ09 | 1.84 | 0.85~2.92 | 19.01 | 0.47 | 0.24~0.80 | 25.98 | 5.22 | 1.36~13.02 | 47.88 |
| | BJ10 | 1.76 | 0.95~2.76 | 24.18 | 0.43 | 0.10~0.79 | 29.22 | 2.8 | 0.48~7.50 | 54.96 |
| Fe | GS09 | 17.59 | 11.45~25.22 | 15.67 | 4.71 | 1.73~8.59 | 25.71 | 72.6 | 25.19~138.38 | 31.09 |
| | GS10 | 17.22 | 11.34~25.49 | 15.4 | 4.67 | 2.61~8.67 | 21.27 | 72.95 | 19.09~143.53 | 32.6 |
| | BJ09 | 16.19 | 11.84~22.20 | 12.67 | 4.29 | 1.44~7.57 | 23.09 | 45.05 | 11.32~99.06 | 42.26 |
| | BJ10 | 18.71 | 12.72~27.48 | 15.2 | 4.58 | 1.36~7.71 | 23.4 | 27.9 | 3.55~69.23 | 53.01 |
| Mn | GS09 | 4.68 | 2.60~7.25 | 21.59 | 1.25 | 0.52~2.34 | 28.67 | 19.12 | 6.33~38.32 | 33.41 |
| | GS10 | 4.77 | 2.40~7.97 | 24.12 | 1.3 | 0.59~2.08 | 27.28 | 19.95 | 3.97~41.02 | 33.43 |
| | BJ09 | 4.49 | 2.95~7.10 | 18.17 | 1.19 | 0.45~2.28 | 28.41 | 12.54 | 3.24~28.42 | 42.53 |
| | BJ10 | 5.56 | 3.07~8.41 | 21.47 | 1.35 | 0.37~2.69 | 25.99 | 8.23 | 0.84~22.17 | 55.31 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Zn | GS09 | 12.39 | 8.35~17.41 | 15.94 | 3.32 | 1.46~6.6 | 27.38 | 50.57 | 18.11~96.83 | 29.5 |
| | GS10 | 13.34 | 8.49~20.69 | 16.89 | 3.62 | 1.64~6.64 | 23.83 | 55.73 | 8.51~101.62 | 31.2 |
| | BJ09 | 16.54 | 10.73~23.09 | 13.49 | 4.39 | 1.88~8.15 | 25.5 | 46.81 | 11.23~115.30 | 44.63 |
| | BJ10 | 21.17 | 13.06~29.15 | 15.42 | 5.17 | 1.72~8.65 | 23.68 | 31.24 | 3.98~78.59 | 50.29 |
| K | GS09 | 3.35 | 2.36~4.32 | 11.74 | 0.9 | 0.39~1.67 | 22.23 | 13.88 | 5.07~25.61 | 29.45 |
| | GS10 | 3.24 | 2.44~4.21 | 10.34 | 0.86 | 0.52~1.49 | 18.07 | 13.65 | 2.52~25.85 | 30.27 |
| | BJ09 | 3.22 | 2.45~4.05 | 8.87 | 0.85 | 0.37~1.44 | 21.07 | 9.03 | 2.24~19.31 | 39.51 |
| | BJ10 | 3.72 | 2.96~4.70 | 9.8 | 0.91 | 0.23~1.44 | 19.97 | 5.81 | 0.91~17.30 | 54.16 |
| Mg | GS09 | 1.07 | 0.75~1.37 | 11.55 | 0.29 | 0.11~0.51 | 25.16 | 4.48 | 1.53~9.21 | 30.57 |
| | GS10 | 1.04 | 0.68~1.45 | 12.74 | 0.28 | 0.15~0.49 | 21.33 | 4.36 | 0.84~8.43 | 30.69 |
| | BJ09 | 1 | 0.81~1.31 | 9.27 | 0.26 | 0.11~0.51 | 22.63 | 2.87 | 0.78~6.48 | 42.55 |
| | BJ10 | 1.1 | 0.85~1.39 | 9.03 | 0.27 | 0.08~0.44 | 19.98 | 1.66 | 0.23~4.28 | 51.72 |
| P | GS09 | 2.8 | 1.94~3.78 | 13.05 | 0.75 | 0.30~1.30 | 25.47 | 11.62 | 4.55~26.25 | 30.28 |
| | GS10 | 2.69 | 1.83~3.75 | 14.05 | 0.74 | 0.42~1.49 | 22.96 | 11.27 | 2.17~21.45 | 29.73 |
| | BJ09 | 2.66 | 2.02~3.57 | 9.39 | 0.71 | 0.30~1.16 | 22.74 | 7.56 | 2.02~17.31 | 43.58 |
| | BJ10 | 3.26 | 2.67~4.08 | 8.07 | 0.8 | 0.22~1.26 | 19.24 | 4.98 | 0.74~12.22 | 51.3 |

[a] Mineral nutrient concentration, content and yield were expressed as mg/kg, mg/seed and g/ha for micronutrients (Cu, Fe, Mn and Z), and g/kg, g/seed and kg/ha for macronutrients (K, Mg and P), respectively.

**Table 4.** Analysis of variances (ANOVA) and heritability ($h^2$) on seed mineral nutrition concentration, content and yield as well as grain yield (GY) and seed weight (SW) in the RIL population grown in four environments. .

| Trait | ANOVA | Concentration | | Content | | Yield | |
|---|---|---|---|---|---|---|---|
| | | DF | MS | DF | MS | DF | MS |
| GY | Year | | | | | 1 | 101.25 *** |
| | Location | | | | | 1 | 1173.43 *** |
| | Genotypes | | | | | 217 | 5.44 *** |
| | Year×Gen | | | | | 197 | 1.71 *** |
| | Loc×Gen | | | | | 183 | 3.01 *** |
| | Error | | | | | 1090 | 0.29 |
| | $h^2$ | | | | | | 0.59 |
| SW | Year | | | | | 1 | 0.031 *** |
| | Location | | | | | 1 | 0.18 *** |
| | Genotypes | | | | | 217 | 0.010 *** |
| | Year×Gen | | | | | 214 | 0.0016 *** |
| | Loc×Gen | | | | | 214 | 0.0015 *** |
| | Error | | | | | 1406 | 0.00045 |
| | $h^2$ | | | | | | 0.85 |
| Cu | Year | 1 | 10.33 *** | 1 | 0.0050 *** | 1 | 106.77 *** |
| | Location | 1 | 25.67 *** | 1 | 2.00 *** | 1 | 558.41 *** |
| | Genotypes | 217 | 0.73 *** | 217 | 0.064 *** | 215 | 13.99 *** |
| | Year×Gen | 215 | 0.18 *** | 203 | 0.015 *** | 170 | 3.39 *** |
| | Loc×Gen | 214 | 0.14 *** | 203 | 0.011 *** | 162 | 7.20 *** |
| | Error | 1076 | 0.076 | 916 | 0.0037 | 663 | 0.89 |
| | $h^2$ | | 0.77 | | 0.81 | | 0.62 |
| Fe | Year | 1 | 434.14 *** | 1 | 12.07 *** | 1 | 13307.80 *** |
| | Location | 1 | 1.25 | 1 | 17.43 *** | 1 | 222651.19 *** |
| | Genotypes | 217 | 34.82 *** | 217 | 4.42 *** | 216 | 1230.16 *** |
| | Year×Gen | 212 | 5.28 *** | 203 | 1.17 *** | 175 | 407.17 *** |
| | Loc×Gen | 211 | 7.83 *** | 203 | 1.24 *** | 164 | 720.50 *** |
| | Error | 1056 | 2.93 | 944 | 0.45 | 668 | 122.05 |
| | $h^2$ | | 0.81 | | 0.74 | | 0.56 |
| Mn | Year | 1 | 105.12 *** | 1 | 4.14*** | 1 | 449.80 *** |
| | Location | 1 | 29.90 *** | 1 | 0.067 | 1 | 14257.25 *** |
| | Genotypes | 217 | 5.83 *** | 217 | 0.53 *** | 214 | 97.91 *** |
| | Year×Gen | 211 | 0.90 *** | 204 | 0.10 *** | 175 | 29.72 *** |
| | Loc×Gen | 212 | 0.93 *** | 205 | 0.099 *** | 165 | 60.89 *** |
| | Error | 1054 | 0.25 | 965 | 0.035 | 687 | 8.77 |
| | $h^2$ | | 0.85 | | 0.81 | | 0.57 |
| Zn | Year | 1 | 2591.56 *** | 1 | 104.53 *** | 1 | 5991.55 *** |
| | Location | 1 | 12224.06 *** | 1 | 527.18 *** | 1 | 30938.84 *** |
| | Genotypes | 217 | 28.06 *** | 217 | 4.17 *** | 216 | 825.20 *** |
| | Year×Gen | 216 | 4.85 *** | 206 | 0.92 *** | 172 | 282.84 *** |
| | Loc×Gen | 216 | 7.57 *** | 204 | 0.84 *** | 167 | 506.21 *** |
| | Error | 1032 | 2.73 | 948 | 0.35 | 688 | 83.88 |
| | $h^2$ | | 0.78 | | 0.79 | | 0.48 |
| K | Year | 1 | 12.84 *** | 1 | 0.49 *** | 1 | 481.05 *** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Location | 1 | 10.83 *** | 1 | 0.018 | 1 | 6590.60 *** |
| | Genotypes | 217 | 0.49 *** | 217 | 0.12 *** | 215 | 37.50 *** |
| | Year×Gen | 213 | 0.10 *** | 205 | 0.031 *** | 176 | 13.17 *** |
| | Loc×Gen | 213 | 0.16 *** | 206 | 0.027 *** | 167 | 22.93 *** |
| | Error | 998 | 0.038 | 946 | 0.011 | 627 | 2.57 |
| | $h^2$ | | 0.74 | | 0.76 | | 0.54 |
| Mg | Year | 1 | 0.35 *** | 1 | 0.0079 ** | 1 | 73.72 *** |
| | Location | 1 | 0.040 *** | 1 | 0.11 *** | 1 | 811.05 *** |
| | Genotypes | 217 | 0.060 *** | 217 | 0.016 *** | 215 | 4.31 *** |
| | Year×Gen | 213 | 0.0094 *** | 203 | 0.0034 *** | 172 | 1.27 *** |
| | Loc×Gen | 213 | 0.019 *** | 201 | 0.0037 *** | 167 | 2.76 *** |
| | Error | 1063 | 0.0047 | 972 | 0.00098 | 681 | 0.26 |
| | $h^2$ | | 0.77 | | 0.77 | | 0.54 |
| P | Year | 1 | 21.35 **** | 1 | 0.88 *** | 1 | 360.26 *** |
| | Location | 1 | 15.54 *** | 1 | 0.095 *** | 1 | 4727.96 *** |
| | Genotypes | 217 | 0.43 *** | 217 | 0.11 *** | 215 | 26.57 *** |
| | Year×Gen | 215 | 0.083 *** | 204 | 0.025 *** | 172 | 8.86 *** |
| | Loc×Gen | 215 | 0.17 *** | 204 | 0.030 *** | 168 | 21.71 *** |
| | Error | 1057 | 0.037 | 964 | 0.0081 | 694 | 2.21 |
| | $h^2$ | | 0.72 | | 0.74 | | 0.48 |

*, **, *** indicated significant level at p<0.05, 0.01 and 0.001, respectively.

**Table 5.** Correlation coefficients (r) between each two traits for grain yield (GY), seed weight (SW) and seed nutrition concentration, content and yield using LSMEAN values across four environments.

| Concentration | SW | GY | Cu | Fe | Mn | Zn | K | Mg | P |
|---|---|---|---|---|---|---|---|---|---|
| SW | | 0.30** | -0.12 | -0.07 | 0.0047 | 0.067 | -0.036 | 0.13 | 0.081 |
| GY | | | -0.11 | -0.14 | -0.053 | -0.14 | -0.079 | -0.028 | -0.15* |
| Cu | | | | 0.11 | -0.026 | 0.043 | 0.13 | -0.013 | 0.049 |
| Fe | | | | | 0.29** | 0.44** | 0.16* | 0.48** | 0.48** |
| Mn | | | | | | 0.40** | 0.10 | 0.39** | 0.39** |
| Zn | | | | | | | 0.15* | 0.42** | 0.48** |
| K | | | | | | | | 0.063 | 0.48** |
| Mg | | | | | | | | | 0.65** |
| P | | | | | | | | | |

| Content | SW | GY | Cu | Fe | Mn | Zn | K | Mg | P |
|---|---|---|---|---|---|---|---|---|---|
| SW | | 0.29** | 0.52** | 0.76** | 0.60** | 0.77** | 0.86** | 0.85** | 0.84** |
| GY | | | 0.094 | 0.22** | 0.13 | 0.077 | 0.18* | 0.20* | 0.11 |
| Cu | | | | 0.49** | 0.33** | 0.47** | 0.54** | 0.49** | 0.47** |
| Fe | | | | | 0.53** | 0.78** | 0.76** | 0.82** | 0.82** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mn | | | | | 0.66** | 0.62** | 0.68** | 0.65** |
| Zn | | | | | | 0.80** | 0.78** | 0.81** |
| K | | | | | | | 0.81** | 0.88** |
| Mg | | | | | | | | 0.91** |
| P | | | | | | | | |

| Yield | SW | GY | Cu | Fe | Mn | Zn | K | Mg | P |
|---|---|---|---|---|---|---|---|---|---|
| SW | | 0.30** | 0.18* | 0.29** | 0.25** | 0.32** | 0.25** | 0.36** | 0.36** |
| GY | | | 0.69** | 0.83** | 0.76** | 0.86** | 0.86** | 0.89** | 0.88** |
| Cu | | | | 0.62** | 0.56** | 0.68** | 0.66** | 0.65** | 0.66** |
| Fe | | | | | 0.79** | 0.84** | 0.85** | 0.88** | 0.88** |
| Mn | | | | | | 0.77** | 0.77** | 0.81** | 0.80** |
| Zn | | | | | | | 0.81** | 0.88** | 0.87** |
| K | | | | | | | | 0.85** | 0.88** |
| Mg | | | | | | | | | 0.96** |
| P | | | | | | | | | |

* and ** indicated relationship coefficient at $p<0.05$ and $p<0.01$, respectively.

**Table 6.** Summary of stable QTL (sQTL) for all investigated traits across all the environments.

| sQTL | Trait | QTL | Environment | Bin[a] | Position | flanking Markers | LOD | Add.[b] | $R^{2}$ [c] |
|---|---|---|---|---|---|---|---|---|---|
| sQTL1 | MnCC | *qMnCC1-1*, *qMnCC1-2* | GS10, BJ10 | 1.04/1.05 | 113.61-114.61 | umc2112-bnlg1884 | 5.90-6.79 | -(0.42-0.46) | 12.78-14.62 |
| sQTL2 | MnCC | *qMnCC2-1*, *qMnCC2-1* | GS09, GS10 | 2.03/2.04 | 48.01 | bnlg2248-phi083 | 3.88-4.18 | 0.34-0.38 | 10.81-11.09 |
| sQTL3.1 | MgCC | *qMgCC3-1*, *qMgCC3-2* | GS09, GS10 | 3.04 | 101.61-103.71 | mmc0132-umc1504 | 3.64-3.65 | 0.036-0.037 | 7.28-7.94 |
| sQTL3.2 | SW | *qSW3-1*, *qSW3-2*, *qSW3-3* | GS10, GS09, BJ09 | 3.08/3.09 | 255.11-263.11 | umc1320-phi047 | 3.02-3.51 | 0.02-2.06 | 16.66-27.27 |
| sQTL3.3 | KCT | *qKCT3-1*, *qKCT3-2* | GS10, BJ09 | 3.08/3.09 | 260.11-269.31 | phi047-umc1062 | 3.11-4.15 | 0.041-0.095 | 6.22-27.75 |
| sQTL4.1 | MnCC | *qMnCC4-1*, *qMnCC4-2* | GS10, BJ10 | 4.05/4.06 | 107.11-115.51 | umc1346-bnlg2291 | 4.82-5.69 | -(0.41-0.47) | 12.88-15.33 |
| sQTL4.2 | ZnCT | *qZnCT4-1*, *qZnCT4-2* | GS09, BJ09 | 4.07 | 145.91-148.91 | umc1620-umc1194 | 3.08-4.27 | 0.30-0.33 | 7.01-12.23 |
| sQTL5 | ZnCC | *qZnCC5-1*, *qZnCC5-1* | GS09, GS10 | 5.03/5.04 | 66.21-69.01 | phi113-umc1990 | 3.43-4.73 | -(0.57-0.65) | 8.33-8.36 |

[a] Chromosome bins of the marker and position taken from IBM 2008;

[b] Positive and negative values representedYe478 and Wu312, respectively, that carried the allele for an increase of trait value.

[c] $R^{2}$: the percentage of the phenotypic variance explained by a putative QTL

**Table 7.** Summary of chromosome clusters for all investigated traits across four environments.

| Cluster | Bin[a] | Position | Number of QTL | QTL | sQTL | LOD | Add [b] | $R^{2}$ [c] |
|---|---|---|---|---|---|---|---|---|
| CL1.1 | 1.04/1.05 | 113.61-120.61 | 3 | *qMnCC1.1, qMnCC1.2, qMnCT1* | sQTL1 | 3.10-6.79 | -0.46~-0.12 | 11.46-14.62 |
| CL1.2 | 1.07 | 200.21-200.21 | 4 | *qGY1.1, qMnYD1, qZnYD1, qMgYD1* | - | 3.98-4.37 | 0.37~1.98 | 17.80-19.04 |
| CL2.1 | 2.03/2.04 | 46.01-48.01 | 3 | *qMnCC2.1, qMnCC2.2, qPCC2* | sQTL2 | 3.88-4.56 | 0.12~0.38 | 10.61-11.09 |
| CL2.2 | 2.05/2.06 | 92.31-99.81 | 3 | *qGY2, qMgCC2, qCuYD2* | - | 3.01-4.62 | -0.80~0.04 | 6.93-13.68 |
| CL3.1 | 3.04 | 89.21-103.71 | 4 | *qMgCT3, qCuCT3.1, qMgCC3.1, qMgCC3.2* | sQTL3.1 | 3.64-5.77 | 0.02~0.04 | 7.28-18.75 |
| CL3.3 | 3.08/3.09 | 255.11-269.31 | 5 | *qSW3.1, qSW3.2, qSW3.3, qKCT3.1, qKCT3.2* | sQTL3.2, sQTL3.3 | 3.02-4.15 | 0.02~2.66 | 6.22-27.27 |
| CL4.1 | 4.05/4.06 | 107.11-121.11 | 5 | *qGY4, qCuYD4, qMnCC4.1, qMnCC4.2 qMgYD4* | sQTL4.1 | 3.25-5.69 | -0.47~0.68 | 9.61-16.14 |
| CL4.2 | 4.07 | 145.91-148.91 | 6 | *qCuCT4, qZnCT4.1, qZnCT4.2, qMnCT4, qPCT4.2, qFeCT4* | sQTL4.2 | 2.24-4.27 | 0.05~0.34 | 6.99-12.23 |
| CL5.1 | 5.05 | 140.01-148.01 | 5 | *qKCT5, qSW5, qPCT5, qZnCT5, qMgCT* | - | 3.20-4.29 | -0.31~-0.01 | 5.84-17.38 |
| CL5.2 | 5.06 | 171.01-175.01 | 3 | *qZnCC5.4, qZnYD5, qMgCC5* | - | 4.03-6.72 | -7.20~-0.04 | 9.43-12.54 |
| CL6.1 | 6.07/6.08 | 172.01-183.71 | 6 | *qKCT6, qSW6, qPCT6, qMgCT6, qMgCC6, qZnCT6* | - | 3.17-5.23 | 0.02~0.28 | 9.12-19.48 |
| CL7.1 | 7.03 | 113.01-113.01 | 3 | *qSW7, qFeCT7, qZnCT7* | - | 3.46-4.56 | 0.02~0.44 | 10.42-13.78 |

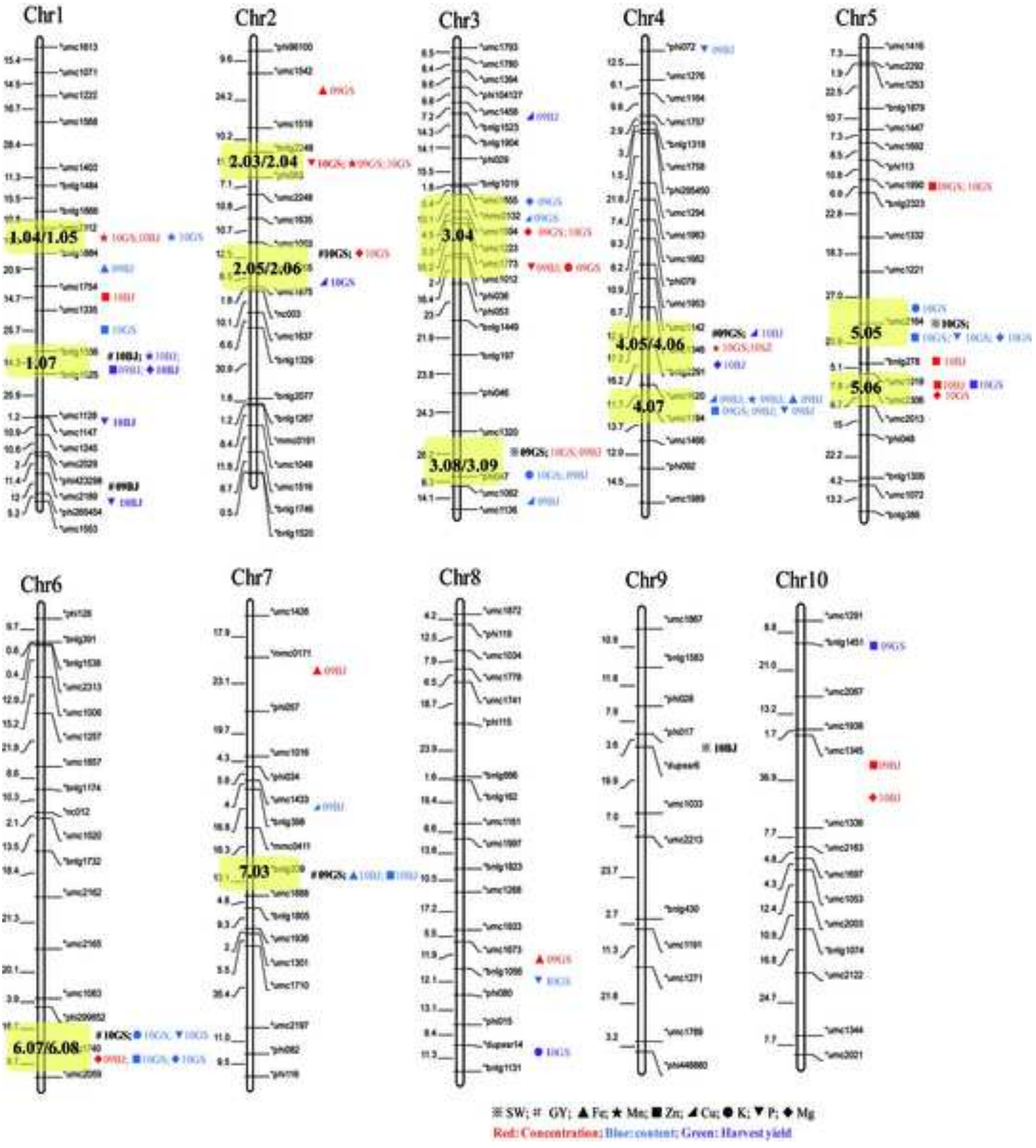
[a] Chromosome bins of the marker and position taken from IBM 2008.

[b] A positive and negative values representedYe478 and Wu312, respectively, that carried the allele for an increase of trait value.

[c] The squared partial correlation coefficient that is the coefficient of determination between the respective QTL and the phenotypic observatio

Figure 1

Click here to download high resolution image



# Supplementary materials

# Comprehensive phenotypic analysis and quantitative trait locus identification for seed mineral concentration, content, and yield in maize grains

Riliang Gu [1#], Fanjun Chen [1#], Bingran Liu [1], Xin Wang [1], Jianchao Liu [1], Pengcheng Li [1], Qingchun Pang [1], Jordon Pace [2], Thomas Lübberstedt [2], Guohua Mi [1], Lixing Yuan [1*]

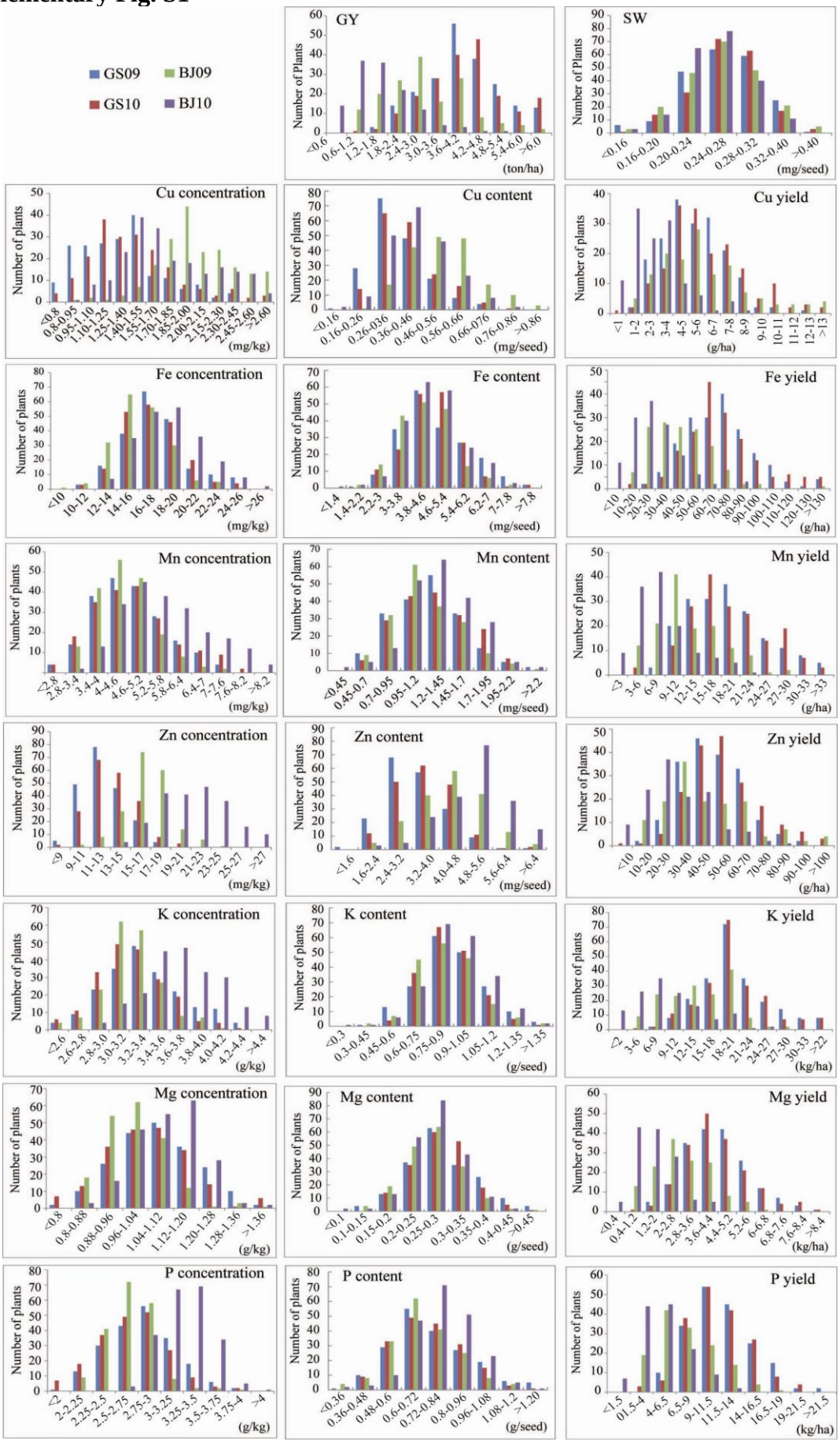
[1] *Department of Plant Nutrition, College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China*

[2] *Department of Agronomy, 1211 Agronomy Hall, Iowa State University, Ames, IA 50011, USA*

*# These authors contributed equally to this work*

** Corresponding author:* Lixing Yuan, Email: yuanlixing@cau.edu.cn; Phone: +86-10-62734424

**Supplementary Fig. S1**



**Supplementary Fig. S1.** Population distributions for grain yield, seed weight and seed nutrient (Cu, Fe, Mn, Zn, K, Mg and P) concentration, content and yield in the Ye478 × Wu312 recombinant inbred lines grown in four environments GS09, GS10, BJ09 and BJ10.

**Supplementary Table S1**. Monthly precipitation (mm) and 18 year-average (from 1996 to 2003) in Beijing location. Data were extracted from http://cdc.cma.gov.cn.

| Environ-ment | Month | | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| BJ09 | 0 | 18.0 | 7.4 | 32.2 | 14.7 | 95.5 | 196.6 | 60.9 | 23.3 | 5.9 | 26.1 | 0 | 480.6 |
| BJ10 | 10.4 | 2.6 | 22.2 | 17.5 | 29.5 | 88.7 | 34.0 | 177.8 | 80.8 | 59.0 | 0 | 0 | 522.5 |
| BJ Average | 4.1 | 6.2 | 13.1 | 26.3 | 32.4 | 78.8 | 141.9 | 105.0 | 51.4 | 31.0 | 20.0 | 2.5 | 512.1 |

**Supplementary Table S2.** LOD threshold value for grain yield (GY), seed weight (SW) and seed nutrient concentration, content and yield. QTL analyses were conducted by composite interval mapping using Windows QTL Cartographer version 2.5 (Model 6) (Wang et al. 2012). Forward regression was performed using a window size of 10 cM and a walking speed of 2 cM. The threshold LOD value was determined with 1000 permutations at P=0.05 level (Churchill and Doerge 1994).

| | Concentration | | | | Content | | | | Yield | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GS09 | GS10 | BJ09 | BJ10 | GS09 | GS10 | BJ09 | BJ10 | GS09 | GS10 | BJ09 | BJ10 |
| GY | 3.0 | - | 2.9 | 2.9 | | | | | | | | |
| SW | - | 3.0 | - | 2.9 | | | | | | | | |
| Cu | - | - | - | - | 2.9 | 3.0 | 3.0 | - | - | 3.0 | 3.0 | 3.0 |
| Fe | 3.0 | - | 2.9 | - | - | 2.9 | 2.9 | 3.0 | - | - | - | - |
| Mn | 3.1 | 3.0 | 3.0 | 3.3 | 3.2 | - | 3.0 | 2.9 | - | - | 3.2 | 3.0 |
| Zn | 3.2 | 3.0 | 2.9 | 3.1 | 3.0 | 3.0 | 3.0 | 3.1 | 2.9 | 3.0 | 3.0 | - |
| K | 3.0 | - | - | - | - | 3.1 | 3.0 | - | - | 2.9 | - | - |
| Mg | 3.0 | 2.9 | 3.0 | 3.1 | - | 3.0 | 3.0 | - | - | - | - | 3.0 |
| P | - | 3.1 | 3.0 | - | 3.1 | 3.1 | 2.9 | 3.0 | - | - | 3.3 | 3.0 |

–: no QTL was detected for this trait.

References

Wang S, Basten CJ, Zeng ZB (2012) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963-971.

**Supplementary Table S3.** QTLs detected for grain yield, seed weight and seed nutrient concentration, content and yield in four environments GS09, GS10, BJ09 and BJ10.

| Environ-ment | Trait | QTL [a] | Bin [b] | Peak | surrouding Markers | LOD [c] | Add [d] | $R^{2,}$ % |
|---|---|---|---|---|---|---|---|---|
| GS09 | GY | *qGY2* | 2.05/2.06 | 92.31 | umc1003-umc1065 | 4.62 | -0.44 | 13.68 |
| | SW | *qSW3.2* [e] | 3.08/3.09 | 263.11 | umc1320-phi047 | 3.02 | 2.06 | 16.66 |
| | CuCT | *qCuCT3.1* | 3.04 | 95.61 | mmc0132-umc1504 | 4 | 0.04 | 10.45 |
| | | *qCuCT7* | 7.02 | 70.91 | umc1433-bnlg398 | 3.49 | 0.03 | 7.11 |
| | FeCC | *qFeCC2* | 2.02 | 27.61 | umc1542-umc1518 | 3.9 | 1.05 | 14.39 |
| | | *qFeCC8* | 8.08 | 148.11 | umc1673-bnlg1056 | 3.28 | 0.69 | 6.05 |
| | MnCC | *qMnCC2.1* | 2.03/2.04 | 48.01 | bnlg2248-phi083 | 3.88 | 0.34 | 11.09 |
| | ZnCC | *qZnCC5.1* | 5.03/5.04 | 66.21 | phi113-umc1990 | 3.43 | -0.57 | 8.36 |
| | ZnCT | *qZnCT4.1* | 4.07 | 148.91 | umc1620-umc1194 | 4.27 | 0.33 | 12.23 |
| | ZnYD | *qZnYD10* | 10.02/10.03 | 8.81 | bnlg1451-umc2067 | 3.02 | 3.85 | 6.56 |
| | KCT | *qKCC3* | 3.04 | 121.51 | umc1773-umc1012 | 3.63 | -0.17 | 18.75 |
| | MgCC | *qMgCC3.1* | 3.04 | 101.61 | mmc0132-umc1504 | 3.65 | 0.03 | 7.94 |
| GS10 | SW | *qSW3.1* | 3.08/3.09 | 259.11 | umc1320-phi047 | 3.35 | 0.02 | 20.82 |
| | | *qSW5* | 5.05 | 144.01 | umc2164-bnlg278 | 3.2 | -0.01 | 5.84 |
| | | *qSW6* | 6.07 | 176.01 | phi299852-bnlg1740 | 5.23 | 0.02 | 13.98 |
| | CuYD | *qCuYD2* | 2.06 | 99.81 | umc1065-umc1875 | 3.68 | -0.80 | 10.01 |
| | MnCC | *qMnCC1.1* | 1.04/1.05 | 113.61 | umc2112-bnlg1884 | 5.9 | -0.42 | 12.78 |
| | | *qMnCC2.2* | 2.03/2.04 | 48.01 | bnlg2248-phi083 | 4.18 | 0.38 | 10.81 |
| | | *qMnCC4.1* | 4.05/4.06 | 115.51 | umc1346-bnlg2291 | 4.82 | -0.41 | 12.88 |
| | ZnCC | *qZnCC5.2* | 5.04 | 69.01 | umc1990-bnlg2323 | 4.73 | -0.65 | 8.33 |
| | ZnCT | *qZnCT1* | 1.06/1.07 | 178.51 | umc1335-bnlg1556 | 3.37 | -0.49 | 31.35 |
| | | *qZnCT5* | 5.05 | 147.01 | umc2164-bnlg278 | 4.29 | -0.31 | 12.28 |
| | | *qZnCT6* | 6.07/6.08 | 183.71 | bnlg1740-umc2059 | 3.17 | 0.28 | 9.12 |
| | ZnYD | *qZnYD5* | 5.06 | 171.01 | umc1019-umc2306 | 4.03 | -5.38 | 9.43 |
| | KCT | *qKCT3.1* | 3.09 | 269.31 | phi047-umc1062 | 3.11 | 0.04 | 6.22 |
| | | *qKCT5* | 5.04/5.05 | 140.01 | umc1221-umc2164 | 4.25 | -0.07 | 17.38 |
| | | *qKCT6* | 6.07 | 172.01 | phi299852-bnlg1740 | 3.98 | 0.07 | 19.48 |
| | KYD | *qKYD8* | 8.09 | 203.61 | dupssr14-bnlg1131 | 3.3 | -1.27 | 9.16 |
| | MgCC | *qMgCC2* | 2.05/2.06 | 95.31 | umc1003-umc1065 | 3.01 | 0.04 | 6.93 |
| | | *qMgCC3.2* | 3.04 | 103.71 | umc1504-umc1223 | 3.64 | 0.04 | 7.28 |
| | | *qMgCC5* | 5.06 | 175.01 | umc1019-umc2306 | 4.05 | -0.04 | 9.77 |
| | MgCT | *qMgCT3* | 3.04 | 89.21 | umc1655-mmc0132 | 5.77 | 0.02 | 12.15 |
| | | *qMgCT5* | 5.05 | 148.01 | umc2164-bnlg278 | 4.27 | -0.02 | 13.32 |
| | | *qMgCT6* | 6.07/6.08 | 177.71 | bnlg1740-umc2059 | 5.17 | 0.02 | 9.95 |
| | PCC | *qPCC2* | 2.03/2.04 | 46.01 | bnlg2248-phi083 | 4.56 | 0.12 | 10.61 |
| | PCT | *qPCT5* | 5.05 | 144.01 | umc2164-bnlg278 | 3.68 | -0.05 | 7.3 |
| | | *qPCT6* | 6.07 | 176.01 | phi299852-bnlg1740 | 3.71 | 0.06 | 9.83 |
| | | *qPCT8* | 8/08/8.09 | 160.01 | bnlg1056-phi080 | 3.57 | -0.05 | 6.8 |
| BJ09 | GY | *qGY1.2* | 1.08/1.1 | 257.11 | phi423298-umc2189 | 3.81 | 0.42 | 11.71 |
| | SW | *qSW3.3* | 3.08/3.09 | 255.11 | umc1320-phi047 | 3.51 | 2.66 | 27.27 |
| | CuCT | *qCuCT3.2* | 3.09/3.1 | 284.61 | umc1062-umc1136 | 3.07 | 0.04 | 9.87 |
| | | *qCuCT4* | 4.07 | 145.91 | umc1620-umc1194 | 3.24 | 0.04 | 6.99 |
| | CuYD | *qCuYD3* | 3.02/3.03 | 36.31 | umc1458-bnlg1523 | 3.41 | -1.10 | 13.48 |
| | FeCC | *qFeCC7* | 7.01 | 17.91 | mmc0171-phi057 | 3.9 | 0.69 | 8.03 |
| | FeCT | *qFeCT1* | 1.05/1.06 | 145.91 | bnlg1884-umc1754 | 3.66 | -0.34 | 11.88 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *qFeCT4* | 4.07 | 147.91 | umc1620-umc1194 | 4.01 | 0.34 | 11.18 |
| | MnCT | *qMnCT4* | 4.07 | 146.91 | umc1620-umc1194 | 4.1 | 0.11 | 9.73 |
| | ZnCC | *qZnCC10* | 10.03 | 45.71 | umc1345-umc1336 | 3.38 | 0.71 | 8.16 |
| | ZnCT | *qZnCT4.2* | 4.07 | 145.91 | umc1620-umc1194 | 3.08 | 0.30 | 7.01 |
| | ZnYD | *qZnYD1* | 1.07 | 200.21 | bnlg1556-bnlg1025 | 3.98 | 1.99 | 17.8 |
| | KCT | *qKCT3.2* | 3.08/3.09 | 260.11 | umc1320-phi047 | 4.15 | 0.10 | 27.75 |
| | MgCC | *qMgCC6* | 6.07/6.08 | 180.71 | bnlg1740-umc2059 | 3.29 | 0.03 | 9.57 |
| | PCC | *qPCC3* | 3.04 | 118.51 | umc1773-umc1012 | 3.41 | -0.09 | 13.99 |
| | PCT | *qPCT4-1* | 4.01 | 2.01 | phi072-umc1276 | 5.74 | -0.06 | 14.68 |
| | | *qPCT4-2* | 4.07 | 146.91 | umc1620-umc1194 | 4.06 | 0.05 | 9.85 |
| | GY | *qGY1.1* | 1.07 | 200.21 | bnlg1556-bnlg1025 | 4.31 | 0.38 | 19.04 |
| | | *qGY4* | 4.05 | 112.11 | umc1142-umc1346 | 3.83 | 0.31 | 11.31 |
| | | *qGY9* | 9.02 | 30.61 | phi017-dupssr6 | 3.4 | 0.28 | 9.24 |
| | SW | *qSW7* | 7.03 | 113.01 | bnlg339-umc1888 | 4.44 | 0.02 | 13.78 |
| | CuYD | *qCuYD4* | 4.05 | 112.11 | umc1142-umc1346 | 5.11 | 0.68 | 16.14 |
| | FeCT | *qFeCT7* | 7.03 | 113.01 | bnlg339-umc1888 | 3.46 | 0.36 | 10.42 |
| | MnCC | *qMnCC1.2* | 1.04/1.05 | 114.61 | umc2112-bnlg1884 | 6.79 | -0.46 | 14.62 |
| | | *qMnCC4.2* | 4.05 | 107.11 | umc1142-umc1346 | 5.69 | -0.47 | 15.33 |
| | MnCT | *qMnCT1* | 1.04/1.05 | 120.61 | umc2112-bnlg1884 | 3.1 | -0.12 | 11.46 |
| BJ10 | MnYD | *qMnYD1* | 1.07 | 200.21 | bnlg1556-bnlg1025 | 3.98 | 1.99 | 17.8 |
| | ZnCC | *qZnCC1* | 1.06 | 148.81 | umc1754-umc1335 | 4.6 | -0.91 | 7.64 |
| | | *qZnCC5.3* | 5.05 | 157.01 | umc2164-bnlg278 | 4.78 | -1.56 | 22.44 |
| | | *qZnCC5.4* | 5.06 | 171.01 | umc1019-umc2306 | 6.72 | -1.16 | 12.54 |
| | ZnCT | *qZnCT7* | 7.03 | 113.01 | bnlg339-umc1888 | 4.56 | 0.44 | 13.65 |
| | MgCC | *qMgCC10* | 10.03 | 71.71 | umc1345-umc1336 | 3.99 | -0.06 | 38.14 |
| | MgYD | *qMgYD1* | 1.07 | 200.21 | bnlg1556-bnlg1025 | 4.37 | 0.38 | 18.59 |
| | | *qMgYD4* | 4.05/4.06 | 121.11 | umc1346-bnlg2291 | 3.25 | 0.29 | 9.61 |
| | PYD | *qPYD1-1* | 1.07 | 238.61 | umc1147-umc1245 | 3.19 | -0.99 | 12.43 |
| | | *qPYD1-2* | 1.11 | 265.51 | umc2189-phi265454 | 3.39 | 0.89 | 9.32 |

[a] QTL nomenclature: q + Trait (Capital letter) + Chromosome + QTL number (McCouch et al. 1997).

[b] Chromosome bin of the marker and position taken from IBM 2008

[c] Add.: additive effects; Positive and negative values represented Ye478 and Wu312, respectively, that carried the allele for an increase of trait value.

[d] $R^2$: the percentage of the phenotypic variance explained by a putative QTL

[e] QTL within a stable QTL region (Table 4) were highlighted by gray background